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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/840,182	05/05/2004	Michael D. Cleary	STAN-304	7494

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EXAMINER	
PANDE, SUCHIRA	
ART UNIT	PAPER NUMBER
1637	

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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/840,182	CLEARY ET AL.	
	Examiner	Art Unit	
	SUCHIRA PANDE	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *06 March 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☐ Claim(s) *5,7-27 and 33-37* is/are pending in the application.

4a) Of the above claim(s) *8,9,12,14-17,20-22 and 24-27* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *5, 7, 10-11, 13, 18-19, 23, 33-37* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Claim Status

1. Amendment filed on March 6, 2008 is acknowledged. Claims 1-4, 6, 28-32 and 38-41 are cancelled. Applicant has amended claims 7, 10, 23, and 33; withdrawn claims 8-9, 12, 14-17, 20-22, 24-27; consequently claims 5, 7, 10-11, 13, 18-19, 23, 33-37 that are active will be examined in this action.

Response to Arguments

Re 112 enablement rejection

2. Applicant has cancelled broad claim 1 that referred to a purine or pyrimidine analog having a reactive moiety not normally present in RNA in claim 1. This overcomes the scope of enablement issue raised in last office action. Hence the 112 1st rejections of claims 1-7, 10-11, 13, 18-19, 23, 28, 31, 33-37 is withdrawn.

Re rejection of base claim 5

3. Applicant's arguments filed March 6, 2008 have been fully considered but they are not persuasive. Applicant argues two points:

1. 4 thiouridine taught by art is not an analog of uracil.

2. UPRT activity is not present in mammals.

Examiner's response to point no 1: Applicant has not defined uracil analogs in the specification. 4 thiouridine taught by Melvin et al. is a substrate that has a thiol moiety not normally present in RNA and is being considered an analog of uracil. The rationale used by Examiner is as follows: The cited art taught uridine analog specifically 4 –thiouridine. There are two possible

biochemical pathways via which the uptaken 4-thiouridine gets converted to corresponding uridine monophosphate which in turn gets incorporated into RNA thus producing thio labeled RNA.

Scenario 1, when the cell takes up 4-thiouridine then once inside the cell 4-thiouridine is hydrolyzed by enzyme uridine nucleosidase and converted to 4 - thiouracil. 4 thiouracil will get converted to 4-thio-UMP by uracil phosphoribosyltransferase.

Scenario 2, once inside the cell 4 thiouridine can form 4-thio-UMP by action of enzyme uridine kinase. Either way 4-thouridine gets converted to 4-thio-UMP, which in turn gets incorporated in RNA. Hence Examiner considers 4 – thiouridine to be a uracil analog. (Shibata et al. 1982 see attached abstract evidence the fact that 4 thiouridine indeed does get taken up by cell and can be converted to 4-thio UMP via the two biochemical pathways).

Examiner's response to point no 2. Active steps of independent claim 5, requires that the uracil analog (4 thiouridine) be taken up by cell and that 4-thiouridine be converted to 4-thio-UMP, which in turn be incorporated into RNA comprising thiol moiety. Since RNA produced by Melvin et al. is labeled with thio moiety then it must have happened by either of the two routes. Thus cited art is still applicable to independent claim 5 because the mammalian cells used by Melvin et al certainly contain phosphoribosyltransferase or nucleoside kinase otherwise one would not have observed the formation of thio labeled RNA. Thus rejection of claim 5 and all the claims that depend from claim are being maintained over cited art.

Re rejection of base claim 33

4. Regarding independent claim 33, the cited art teaches the active steps of the method namely contacting a cell with a uracil analog having thiol moiety, the conversion of uracil analog into corresponding uridine monophosphate such that uracil analog is incorporated into RNA synthesized by said cell. Hence inherently cells used by Melvin et al. are capable of taking up the uracil analog and the conversion of uracil analog into corresponding uridine monophosphate such that uracil analog is incorporated into RNA synthesized by said cell.

Applicant is correctly arguing that mammalian cells do not have UPRT. Hence Examiner's conclusion that the cells taught by Melvin et al. must inherently have UPRT is incorrect. The labeling of RNA must be occurring through the scenario 2 described above using the (nucleoside kinase) uridine kinase. Therefore rejection of independent claim 33 and other claims that depend from claim 33 over previously cited art under 102 and 103 is being withdrawn.

New grounds of rejection are being introduced to teach the UPRT limitation of claim 33.

Any pending rejection that is not reiterated below is withdrawn.

Claim interpretation

5. Applicant has not provided any specific definition of “tag” or “conjugating” in the specification. Examiner is interpreting “tag” to mean any means that may be used to bind RNA through said thiol moiety. “Conjugating” is being broadly interpreted as any mechanism by which the thiol moiety can be bound to the “tag”. This binding mechanism includes both, covalent or non-covalent binding.

Claim Rejections - 35 USC § 102

6. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

7. Claims 33-34 are rejected under 35 U.S.C. 102(b) as being anticipated by Shibata et al. (1982) Plant and Cell Physiol. Vol. 23(3) : 365-374.

Regarding claim 33, Shibata et al. teaches a method of biosynthetically labeling RNA in a cell of interest (see title and abstract where labeling of RNA from seedlings of Radish with many 4-thio containing compounds is taught), the method comprising:

contacting said cell (radish seedlings) with a uracil analog having a reactive thiol moiety not normally present in RNA (see abstract where labelling with [2-^{14}C]-4-thiouracil is taught),

wherein said cell comprises a uracil phosphoribosyltransferase (UPRT) that can convert said uracil analog to the corresponding uridine monophosphate (see abstract);

wherein said uracil analog is incorporated into RNA synthesized by said cell (see page 368 par. 1 where Hg-cellulose affinity chromatography is taught for isolation of thiolated RNA).

Regarding claim 34, Shibata et al. teaches wherein sequences encoding said UPRT are operably linked to a promoter that is active or can be activated in

said cell. (By teaching when precursors C^{14} labeled 4 thiouracil are fed to cells its taken up by cells and thio residues are incorporated into RNA Shibata et al. inherently teach these radish cells contain sequences encoding said UPRT that are operably linked to a promoter that is active or can be activated in said cell).

Claim Rejections - 35 USC § 103

8. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

9. Claims 5, 7, 10-11, 13, 18-19 are rejected under 35 U.S.C. 103(a) as being unpatentable over Melvin et al. (1978) Eur. J. Biochem. 92:373-379 and as evidenced by Woodward et al. (1998) Analytical biochemistry 171:166-172 (both references provided by applicant in IDS) in view of Rana (P.G. Pub

2004/0175732 filed on November 17, 2003 with a priority date of November 15, 2002) as evidenced by Diamandis and Christopoulos (1991) Clin Chem 37 (5) pp 625-636 (provided by Applicant in IDS).

Regarding claim 5, Melvin et al. teach, A method of biosynthetically labeling RNA in a cell of interest (baby hamster kidney cell line BHK-21 was used a cell of interest), the method comprising:

contacting said cell with a uracil analog having a reactive thiol moiety not normally present in RNA (See Fig. 4 on page 377 and read Fig. legend where 4 thiouridine, 4- TU is not normally present in rRNA and mRNA of these cells is used to label cells such that RNA that gets labeled with thiol moiety. The 4 TU is provided to cells through addition to culture media—Fig. 1),

wherein said cell comprises a phosphoribosyltransferase or nucleoside kinase operably linked to a promoter that can be activated in said cell, and that can specifically incorporate said uracil analog into the corresponding nucleotide (4 thio- uridine monophosphate);

wherein said uracil analog is incorporated into RNA comprising said thiol moiety (the fact the RNA obtained from this cell is labeled with thiol moiety indicated that the cell does contain either phosphoribosyltransferase or nucleoside kinase that can convert said uracil analog to the corresponding nucleotide uridine monophosphate which in turn gets incorporated into RNA);

obtaining RNA comprising said thiol moiety from said cell (by teaching RNA extraction from cultured cells grown in presence of 4-TU—Fig. 1 legend, Melvin et al. teach obtaining RNA comprising said thiol moiety from said cell);

Thus regarding claim 5 Melvin et al. teach all aspects of this claims but does not teach *conjugating a small molecule binding partner to said thiol moiety.*

Regarding claim 5, Rana teaches: *conjugating a tag to said thiol moiety.*(see page 1 par. 0004 where Rana teaches addition of tags such as biotin, psoralen-biotin and 4-thiobiotin to isolated RNA. See Fig. 8 D and page 6, par. 0065 where Rana teaches biotinylated RNA molecule labeled with 4-thio uridine or 6-thioguanosine. The thiol moiety can be used to bind biotin is evidenced by teachings of Diamandis and Christopoulos (1991) Clin Chem 37 (5) pp 625-636 ---ref provided by applicant in IDS where on page 630 last par. they teach ---- 4 thiouridine containing nucleic acid is taught to be biotinylated by using haloacetamido derivative of biotin e.g. iodoacetyl-LC-biotin that reacts with thiol group on the nucleic acid to result in biotinylated nucleic acid. See page 629 par. 4. where they state " another useful class of biotinylated nucleotides consists of nucleotides that contain S-S within the structure of the linker arm. These "releasable" nucleotide analogs can be used in applications where the nucleic acid needs to be released after it's binding to streptavidin.

Regarding claim 7, Rana teaches wherein *said small molecule binding partner* is biotin. (see page 1, par. 0004 where small molecule binding partner biotin is taught as a tag).

Regarding claim 10, Rana teaches *method further comprising the step of binding a specific binding partner to said small molecule binding partner.* (see page 6, par. 0065 where the step of binding streptavidin, a specific binding partner to said *small molecule binding partner* namely biotin is taught).

Regarding claim 11, Rana teaches *wherein said specific binding partner is conjugated to an insoluble substrate for affinity chromatography, and wherein said biosynthetically labeled RNA is separated from non-labeled RNA.* (see page 6, par. 0065 where use of streptavidin coated beads to separate biotin labeled RNA from total RNA is taught. Here specific binding partner (streptavidin) is conjugated to an insoluble substrate (beads) for affinity chromatography, and wherein said biosynthetically labeled RNA is separated from non-labeled RNA.

Regarding claim 13, Rana teaches *wherein said separated RNA is amplified*. (see page 6, par. 0069 where amplification of RNA by reverse transcription is taught).

Regarding claim 18, Rana teaches wherein *said specific binding partner is conjugated to a detectable label.* (see page 13, par. 0145 where avidin and streptavidin conjugated to different detectable labels magnetic particles, superparamagnetic microspheres are taught. Further Rana teaches custom synthesis of beads when biotin/avidin or biotin/streptavidin system is used. Thereby Rana teaches the specific binding partner could be labeled with any other desired detectable label)

Regarding claim 19, Rana teaches *wherein said detectable label is a fluorochrome, radiolabel, heavy metal label, or enzyme conjugate*. (see page 13, par. 0141 where detectable labels such as fluorochrome such as fluorescein, radiolabel such as ^{32}P etc are taught)

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Rana in the method

of labeling RNA taught by Melvin et al. The motivation to do so is provided to one of ordinary skill by teaching of both Melvin et al. and Rana. Melvin et al. teach a method of labeling RNA with thiol groups. This thiolated RNA can be further tagged using the biotinylation techniques taught by Rana.

Rana states " -----biotinylated miRNAs can be made incorporating 4-thio uridine or thymidine or 6-thio guanosine in the miRNA sequence, as illustrated in ---.The 4-thio uridine, 4-thio thymidine or 6-thio guaosine can be incorporated into the RNA sequence of the miRNA according to methods known in the art e.g. as described in --------. Total mRNA is isolated from the cell and streptavidin coated beads added to the extract to enrich for the miRNA target complexes---- (see page 6 par. 0065).

Thus Rana explicitly teaches one of ordinary skill that thiolated RNA produced by incorporation of 4-thio uridine, 4-thio thymidine or 6-thio guanosine is a substrate to which biotin tag can be conjugated. Once thiolated RNA is tagged with biotin now the streptavidin chemistry can be exploited for detection of various downstream products resulting from use of labeled RNA. In view of the teaching by Melvin et al. page 374 par. 5 where DTT based elution of thiolated RNA is taught, one of ordinary skill recognizes that the conjugation of tags to RNA via thiol moiety gives the additional advantage that the final product can be released from the tag using dithiothreitol based /cleavage or elution.

10. Claim 23 is rejected under 35 U.S.C. 103(a) as being unpatentable over Melvin et al. (1978) in view of Rana (priority date of November 15, 2002) as

applied to claim 5 above further in view of Tiraby et al. (1998) FEMS Microbiology Letters 167 pp 41-49.

Regarding claim 23, Melvin et al. in view of Rana et al. teach method of claim 5, but Melvin et al and Rana et al. are silent about the nature of the promoter.

Regarding claim 23, Tiraby et al. teach wherein bacterial UPRT gene referred to as upp is cloned under control of constitutive EM7 promoter in plasmid pUT847 (see page43 section 2.2 plasmid construction and fig. 2 on page 44 where pUT847 is graphically depicted).

It would have been prima facie obvious to one of ordinary skill to practice the method of Tiraby et al. in the method of Melvin et al. and Rana et al. at the time the invention was made. The motivation to do so is provided by Tiraby et al. who state “The two---and the individual---and *upp* (UPRT gene of *E.coli*) genes were placed under the control of a constitutive promoter to avoid the complex response elicited by some carbon and nitrogen compounds on the natural promoters of these genes." (see page 45 last part of results section 3.1). Thus prior art provides the explicit motivation to one of ordinary skill to have UPRT gene cloned under the control of constitutive promoter to avoid the complex response elicited by some carbon and nitrogen compounds on the natural promoters of these genes.

11. Claim 35, 37 is rejected under 35 U.S.C. 103(a) as being unpatentable over Shibata et al. as applied to claims 33 and 34 above further in view of Al-

Anouti et al. (January 2003) Biochemical and Biophysical Research Communications vol. 302: pp. 316-323—Previously provided to applicant).

Regarding claim 35, Shibata et al teach method of claim 33. But do not teach wherein said sequences encoding said UPRT are exogenous to the cell of interest.

Regarding claim 35, Al-Anouti et al. teaches *wherein said sequences encoding said UPRT are exogenous to the cell of interest.* (see page 317, par. 4 where pUC19UPRT plasmid is taught. These pUC based plasmids can be propagated in *E.coli.* Thus Al-Anouti et al. teaches UPRT gene from *T.gondii* is exogenous to the cell of interest.)

Regarding claim 37, Al-Anouti et al. teaches *wherein said UPRT is Toxoplasma gondii UPRT or a functional derivative thereof.* (see page 316 title and abstract where *Toxoplasma gondii* uracil phosphoribosyltransferase (TgUPRT) is taught).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Al-Anouti et al. in the method of labeling RNA taught by Shibata et al. The motivation to do so is provided to one of ordinary skill by teachings of art and Al-Anouti et al.

One of ordinary skill knows that if plasmids containing the gene of interest can be introduced into a cell of interest where the enzyme encoded by the plasmid borne gene is expressed then one has a means of exploiting the enzymatic activity of this exogenous gene in the environment of the new host cells. Art also teaches that mammalian cells lack an endogenous UPRT gene.

Al-Anouti et al. teaches plasmids containing TgUPRT gene that can be expressed in E.coli as well as Human Foreskin Fibroblasts. Having bacterial shuttle vector as well as mammalian vector now enables on of ordinary skill to introduce these mammalian vectors into mammalian cells that lack endogenous UPRT. By combining the method of Al-Anouti et al. in the method of Shibata et al., one of ordinary skill in the art has reasonable expectation of success in being able to thiolate newly synthesized RNA from cells such as mammalian cells which lack endogenous UPRT enzyme. This provides one of ordinary skill a means to thiol label newly synthesized RNA in mammalian cells, conjugate a tag to the thiolated RNA and use the tagged RNA for their desired intent.

12. Claim 36 is rejected under 35 U.S.C. 103(a) as being unpatentable over Shibata et al. as applied to claims 33 above further in view Maddry et al. (US pat. 5,561,225 Oct 1, 1996) as evidenced by Chan (US Pat. 6,403,311 B1 filed Aug 13, 1999) and evidenced by Diamandis and Christopoulos (1991) Clin Chem 37 (5) pp 625-636 (provided by Applicant in IDS).

Regarding claim 36, Shibata teaches the method of claim 33 and teach use of 4-thio Uracil as well as teach [2-^{14}C]-4-thiouracil but do not teach uracil analog 2, 4 dithiouracil.

Regarding claim 36, Maddry et al. teach 2, 4- dithiouracil as a uracil analog (see col. 4 line 3) along with a whole series of Purine and pyrimidine analogs that are made from nucleosides, which contain sulfonate and sulfonamide linkages.

Regarding 2, 4- dithiouracil one of ordinary skill in the art is taught by art that it is an analog that has the characteristic energy emission pattern of a light emitting compound (as evidenced by Chan US pat. 6,403,311 B1 issued Jun 11, 2002 . See col. 11 lines 32-42)

It would have been prima facie obvious to one of ordinary skill in the art to use uracil analog 2,4 dithiouracil out of the numerous analogs taught by Maddry et al. in the method of Shibata et al. at the time the invention was made.

The motivation to do so is provided to one of ordinary skill in the art by both the art itself as well as Shibata et al.

Shibata et al. state "since 4 SU-derivatives (4-thio-U) have maximum absorptions near 330 nm, as compared to the maxima near 260nm of typical nucleic acid components, the presence of 4SU- metabolites can be monitored by their characteristic absorptions. But, water soluble flavonoids -----interfere with the measurements of 4SU-metabolites. Recently we reported an Hg-cellulose affinity chromatography for 4SU-containing RNA" (see page 368 par. 1). Thus using 4 thio uracil one is able to affinity purify labeled RNA.

Having an additional thio molecule in 4 thio Uracil would result in RNA being labeled with S-S bond. While one thio group is used to bind to Hg-cellulose affinity column. The presence of S-S bond would allow cleavage by DTT hence easy elution of labeled RNA. Diamandis and Christopoulos state " another useful class of biotinylated nucleotides consists of nucleotides that contain S-S within the structure of the linker arm. These "releasable" nucleotide analogs can be

used in applications where the nucleic acid needs to be released after it's binding to streptavidin" (see page 630 last par).

One of ordinary skill has reasonable expectation of success for labeling RNA using 2,4-dithiouracil as a substrate that when fed to the cells containing UPRTase will be taken up and incorporated into newly synthesized RNA. This is because Shibata et al. show labeling of 4 thio Uracil at position 2 with C^{14} does not interfere with its uptake or incorporation into RNA. So 2,4- dithiouracil should be taken up. Such a labeled RNA will have triple advantages namely: one can use affinity purification; use the S-S bond to bind biotin; and also have a characteristic energy emission pattern of a light emitting compound.

13. Claims 5, 7, 10-11, 13, 18-19 are rejected under 35 U.S.C. 103(a) as being unpatentable over Shibata et al. (1982) Plant and Cell Physiol. Vol. 23(3) : 365-374 in view of Rana (P.G. Pub 2004/0175732 filed on November 17, 2003 with a priority date of November 15, 2002) as evidenced by Diamandis and Christopoulos (1991) Clin Chem 37 (5) pp 625-636 (provided by Applicant in IDS).

Regarding claim 5, Shibata et al. teach a method of biosynthetically labeling RNA in a cell of interest (see title and abstract where labeling of RNA from seedlings of Radish with many 4-thio containing compounds is taught), the method comprising:

contacting said cell (radish seedlings) with a uracil analog having a thiol moiety not normally present in RNA (see abstract where labelling with [2-^{14}C]-4-thiouracil is taught), wherein said cell comprises a phosphoribosyltransferase or

nucleoside kinase (see abstract where uracil phosphoribosyltransferase or uridine kinase are taught)

operably linked to a promoter that can be activated in said cell (see abstract), and that can specifically incorporate said uracil analog into the corresponding nucleotide, and wherein said uracil analog is incorporated into RNA comprising said thiol moiety (see page 368 par. 1 where Hg-cellulose affinity chromatography is taught for isolation of thiolated RNA):

obtaining RNA comprising said thiol moiety from said cell (see page 368 par. 1 where Hg-cellulose affinity chromatography is taught for isolation of thiolated RNA);

Regarding claim 5, Shibata et al. do not teach:

conjugating a small molecule binding partner to said thiol moiety.

Regarding claim 5, Rana teaches: *conjugating a small molecule binding partner to said thiol moiety*.(see page 1 par. 0004 where Rana teaches addition of tags such as biotin, psoralen-biotin and 4-thiobiotin to isolated RNA. See Fig. 8 D and page 6, par. 0065 where Rana teaches biotinylated RNA molecule labeled with 4-thio uridine or 6-thioguanosine. The thiol moiety can be used to bind biotin is evidenced by teachings of Diamandis and Christopoulos (1991) Clin Chem 37 (5) pp 625-636 ---ref provided by applicant in IDS where on page 630 last par. they teach ---- 4 thiouridine containing nucleic acid is taught to be biotinylated by using haloacetamido derivative of biotin e.g. iodoacetyl-LC-biotin that reacts with thiol group on the nucleic acid to result in biotinylated nucleic acid. See page 629 par. 4. where they state " another useful class of biotinylated nucleotides consists

of nucleotides that contain S-S within the structure of the linker arm. These "releasable" nucleotide analogs can be used in applications where the nucleic acid needs to be released after it's binding to streptavidin.

Regarding claim 7, Rana teaches *wherein said small molecule binding partner is biotin*. (see page 1, par. 0004 where small molecule binding partner biotin is taught)

Regarding claim 10, Rana teaches *method further comprising the step of binding a specific binding partner to said small molecule binding partner.* (see page 6, par. 0065 where the step of binding streptavidin, a specific binding partner to said tag biotin is taught).

Regarding claim 11, Rana teaches *wherein said specific binding partner is conjugated to an insoluble substrate for affinity chromatography, and wherein said biosynthetically labeled RNA is separated from non-labeled RNA.* (see page 6, par. 0065 where use of streptavidin coated beads to separate biotin labeled RNA from total RNA is taught. Here specific binding partner (streptavidin) is conjugated to an insoluble substrate (beads) for affinity chromatography, and wherein said biosynthetically labeled RNA is separated from non-labeled RNA.

Regarding claim 13, Rana teaches *wherein said separated RNA is amplified*. (see page 6, par. 0069 where amplification of RNA by reverse transcription is taught).

Regarding claim 18, Rana teaches wherein *said specific binding partner is conjugated to a detectable label.* (see page 13, par. 0145 where avidin and streptavidin conjugated to different detectable labels magnetic particles,

superparamagnetic microspheres are taught. Further Rana teaches custom synthesis of beads when biotin/avidin or biotin/streptavidin system is used. Thereby Rana teaches the specific binding partner could be labeled with any other desired detectable label)

Regarding claim 19, Rana teaches *wherein said detectable label is a fluorochrome, radiolabel, heavy metal label, or enzyme conjugate*. (see page 13, par. 0141 where detectable labels such as fluorochrome such as fluorescein, radiolabel such as ^{32}P etc are taught)

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to combine the teachings of Rana in the method of labeling RNA taught by Shibata et al. The motivation to do so is provided to one of ordinary skill by teaching of both Shibata et al. and Rana. Shibata et al. teach a method of labeling RNA with thiol groups. This thiolated RNA can be further tagged using the biotinylation techniques taught by Rana.

Rana states " -----biotinylated miRNAs can be made incorporating 4-thio uridine or thymidine or 6-thio guanosine in the miRNA sequence, as illustrated in ---.The 4-thio uridine, 4-thio thymidine or 6-thio guaosine can be incorporated into the RNA sequence of the miRNA according to methods known in the art e.g. as described in --------. Total mRNA is isolated from the cell and streptavidin coated beads added to the extract to enrich for the miRNA target complexes---- (see page 6 par. 0065).

Thus Rana explicitly teaches one of ordinary skill that thiolated RNA produced by incorporation of 4-thio uridine, 4-thio thymidine or 6-thio guaosine is

a substrate to which biotin tag can be conjugated. Once thiolated RNA is tagged with biotin now the streptavidin chemistry can be exploited for detection of various downstream products resulting from use of labeled RNA.

Conclusion

14. All claims under consideration 5, 7, 10-11, 13, 18-19, 23, 33-37 are rejected over prior art.

15. Any inquiry concerning this communication or earlier communications from the examiner should be directed to SUCHIRA PANDE whose telephone number is (571)272-9052. The examiner can normally be reached on 8:30 am -5:00 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on 571-272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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June 5, 2008